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RESEARCH ARTICLE

Benefits of hyperbaric oxygen pretreatment for decompression sickness in Bama pigs

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ABSTRACT

Decompression sickness (DCS) occurs when ambient pressure is severely reduced during diving and aviation. Hyperbaric oxygen (HBO) pretreatment has been shown to exert beneficial effects on DCS in rats via heat-shock proteins (HSPs). We hypothesized that HBO pretreatment will also reduce DCS via HSPs in swine models. In the first part of our investigation, six swine were subjected to a session of HBO treatment. HSP32, 60, 70 and 90 were detected, before and at 6, 12, 18, 24 and 30 h following exposure in lymphocytes. In the second part of our investigation, another 10 swine were randomly assigned into two groups (five per group). All swine were subjected to two simulated air dives in a hyperbaric chamber with an interval of 7 days. Eighteen hours before each dive, the swine were pretreated with HBO or air: the first group received air pretreatment prior to the first dive and HBO pretreatment prior to the second; the second group were pretreated with HBO first and then air. Bubble loads, skin lesions, inflammation and endothelial markers were detected after each dive. In lymphocytes, all HSPs increased significantly (P<0.05), with the greatest expression appearing at 18 h for HSP32 and 70. HBO pretreatment significantly reduced all the determined changes compared with air pretreatment. The results demonstrate that a single exposure to HBO 18 h prior to diving effectively protects against DCS in the swine model, possibly via induction of HSPs.

KEY WORDS: Hyperbaric oxygenation, Inflammatory markers, Decompression illness, Swine, Heat-shock proteins

INTRODUCTION

Rapid desaturation of dissolved inert gas in bodily tissues and blood causes decompression sickness (DCS) at lower pressures, which may occur in recreational and military diving, aviation and extra-vehicular activity (EVA) in space (Vann et al., 2011). The symptoms of DCS range from skin lesions and joint pain to effects on the cardiopulmonary and central nervous system which may even cause sudden death (Vann et al., 2011).

Hyperbaric oxygen (HBO) is an essential therapy for DCS, and has a wide application in the treatment of carbon monoxide poisoning, wound healing, cerebral ischemia and other maladies (Weaver, 2014). HBO can also act in a prophylactic manner to prevent DCS (Butler et al., 2006). We have shown that HBO pretreatment 18 h before a simulated air dive significantly decreased the incidence of and mortality from DCS in a rat model (Fan et al., 2010; Ni et al., 2013) and further verified the involvement of heat shock proteins (HSPs) in the model and in a primary cell culture (Ni et al., 2013; Huang et al., 2014). As a moderate oxidative stress, HBO could induce the expression of protective proteins including HSPs, which could directly interfere with oxidative injury and ischemia-like insults, having anti-oxidative, anti-inflammatory and anti-apoptotic consequences.

The aim of this study was to further verify the beneficial effects of HBO pretreatment related to HSP induction in a swine DCS model. Sixteen swine were employed to reveal the expression of HSPs and DCS symptoms following a single exposure to HBO. The changes in the indices for swine DCS, including bubble formation, skin lesions, inflammation and endothelial markers, were determined to reflect the effects of HBO pretreatment on DCS.

MATERIALS AND METHODS

All experimental procedures were carried out in accordance with internationally accepted humane standards (Russell et al., 1959). Ethical clearance for this study was obtained from the Ethics Committee for Animal Experiments of the Naval Medical University, Shanghai, China.

Animals

A total of 16 neutered, 5 month old male Bama swine, *Sus scrofa domestica* Erxleben 1777, were housed individually in the animal husbandry facility of the Naval Medical University. The animals were fed 2% of their body mass daily and water was available *ad libitum*. They were fasted overnight for 12 h before they were anesthetized, and were accustomed to the general laboratory temperature of 23°C, humidity of 50–65% and natural illumination, and used one at a time in the experiments. The animals were weighed before each treatment.

Experimental design

This study consisted of two parts. Experiment 1 was carried out to confirm the expression of HSPs induced by HBO in six swine. Lymphocyte levels of HSP32, 60, 70 and 90 were determined before, and at 6, 12, 18, 24 and 30 h after HBO exposure. Experiment 2 was performed to explore the effects of HBO pretreatment on DCS in 10 swine. Animals were randomly divided into two subgroups (n=5 each) and subjected to the treatment plan shown in Fig. 1. DCS was evaluated after each decompression as described below.

Surgical preparation

In the operating theatre, the animals were placed in a canvas sling and anesthesia was induced by intramuscular injection of 0.05 mg kg^{-1} atropine and (15 min later) 0.1 ml kg^{-1} Sumianxin; 2 mg kg^{-1} propofol was injected through the ear vein and anesthesia

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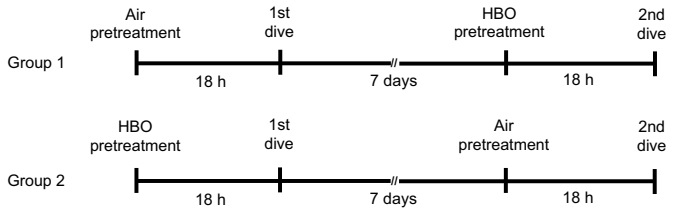

Fig. 1. Profile of the air/HBO pretreatment of swine (experiment 2). Ten swine were randomly divided into two subgroups (n=5 each) and were subjected to two simulated air dives in a hyperbaric chamber with an interval of 7 days. Eighteen hours before each dive, the swine were pretreated with hyperbaric oxygen (HBO) or air. Group 1 received air pretreatment prior to the first dive and HBO pretreatment prior to the second; group 2 was pretreated with HBO first and then air.

was maintained with inhaled isoflurane (6%) via endotracheal intubation using an anesthesia machine (WATO EX-20 Vet, Mindray, Shenzhen, China). The right external jugular vein was exposed and a central venous catheterization set (REF ES-04301, Arrow, Houston, TX, USA) was introduced into the vein for blood sampling. The surgical skin site was sterilized thoroughly with povidone iodine solution and saline before the operation and after suturing; 1 ml of heparinized saline (50 U ml^{-1}) was injected to avoid catheter occlusion and 1.6 MU penicillin was intramuscularly injected to reduce the risk of infection. The animals were sent back to the husbandry facility after complete awakening from anesthesia and no further interventions were made that day.

HBO pretreatment

HBO pretreatment was performed in a 1000 l steel animal chamber (DCW150, Yang Yuan No. 701 Institute, Shanghai, China) on one animal at a time. The animal was secured in the canvas sling and a transparent silicon hood was fixed and sealed around the neck. The chamber was compressed with air to 15 m seawater (msw) and pure oxygen was administered into the hood for 60 min with a 10 min air break. Both compression and decompression were performed at a rate of 2 msw min^{-1} to minimize both any potential discomfort to the animal and temperature changes inside the chamber. The air pretreatment (control) was performed in the same way except air was ventilated into the hood and the chamber was kept at atmospheric pressure. Oxygen concentration in the hood was monitored and maintained above 98% during HBO breathing and between 21% and 23% during the air interval. Chamber temperature and relative humidity were maintained at 22–24°C and 65–75%, respectively.

Determination of HSPs

A 2 ml sample of anticoagulated blood was collected from the indwelling catheter and an equal amount of Sample Diluent Mix (TBD, Tianjin, China) was added. Lymphocytes were separated by Percoll density gradient centrifugation with lymphocyte separation liquid (TBD). Following interfusion with intracellular fixation buffer (eBioscience, San Diego, CA, USA) and permeabilization buffer (eBioscience), lymphocyte membranes were ruptured and fixed. Fluorescent HSP antibodies labeled with FITC or PE (Abcam, Cambridge, UK) and flow cytometry staining buffer (eBioscience) were added, and HSPs in lymphocytes were detected by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA).

Simulated diving

All simulated dives were carried out in the same chamber described above, with one swine at a time. The chamber was pressurized to 40 msw with compressed air over a period of 9 min at an increasing rate from 3 to 6 msw min^{-1}. The pressure was maintained for 35 min before decompression, which was conducted in linear segments of 5 msw min^{-1} between 40 and 30 msw, 4 msw min^{-1} between 30 and 20 msw, 3.3 msw min^{-1} between 20 and 10 msw, and 2.9 msw min^{-1} from 10 msw to the surface. The chamber was frequently ventilated to prevent any decrease in O_2 and accumulation of CO_2. The temperature was controlled between 22 and 24°C with a transient increase to 26°C following compression and a decrease to 20°C before surfacing.

Bubble detection

Bubbles in heart chambers were detected extrathoracically by a 3.5 MHz transducer connected to an ultrasound machine (Mylab 30CV, Esoate, Italy). Detection was repeated at 30 min, 60 min, 90 min, 2 h, 3 h, 4 h and 6 h following surfacing, each lasting 2 min. The aortic root short axis was adjusted into view for detection. In this view, the right ventricular outflow tract, pulmonary artery and aorta were clearly visible. Bubbles in ultrasound images were scored by the Eftedal–Brubakk grading scale (Eftedal and Brubakk, 1997).

Skin lesions

After the swine had surfaced, their skin lesions were thoroughly examined according to methods previously developed by us (Qing et al., 2017). Briefly, the latency and dimension of stage III lesions, the most serious form with maximum lesion area manifesting as purple–red homogeneous, macular lesions, were examined and recorded on a swine-shape figure. Lesions were measured by the palm of a single experimenter, similar to the estimation of burn surface area. The swine body surface area was calculated by the Meeh–Rubner equation area=0.0974×weight$^{2/3}$ (Quiring, 1955).

Detection of inflammation and endothelial markers

Immediately before and 12, 18, 24, 32, 48 h following exposure, a 2 ml blood sample was taken from the catheter in a pro-coagulation tube to segregate serum. Inflammatory indicators including interleukine-8 (IL-8), monocyte chemoattractant protein-1 (MCP-1) and methane dicarboxylic aldehyde (MDA), and endothelial markers including endothelin-1 (ET-1) and vascular cell adhesion molecule-1 (VCAM-1) were detected by the respective ELISA kits (Enzyme-Linked Biotechnology Co., Ltd, Shanghai, China).

Statistical analysis

Changes of HSP expression in lymphocytes following HBO exposure were analyzed by one-group repeated-measures ANOVA. Bubble loads were tested by the generalized estimation equation. ANOVA with two-stage cross-over design data was used to test the effects of pretreatment and stage. Indexes from two simulated dives in each group were compared by paired-samples t-tests. Differences between groups were compared by two independent-samples t-tests. $P \leq 0.05$ was accepted as statistically significant.

RESULTS

The swine weighed 20–25 kg (22.0±0.9 kg, mean±s.d.). In experiment 2, no significant differences in mean mass were found between the two groups before the first or the second pretreatment and dive. Mean body mass increased approximately 7% over the 7 day interval between the two dives, from 21.5±1.0 to 23.0±0.7 kg (P=0.00). All swine survived the experiments.

HSP expression after HBO exposure

All detected HSPs expressed in lymphocytes increased significantly after HBO exposure (experiment 1; P<0.05). The rate of expression

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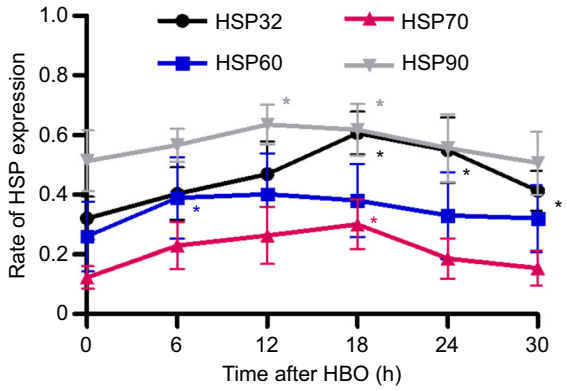

Fig. 2. Heat shock protein (HSP) expression in lymphocytes following HBO exposure. Swine (n=6) were treated with HBO at 15 m seawater (msw) for 60 min with a 10 min air break (experiment 1). The expression of HSP32, 60, 70 and 90 in lymphocytes was detected by flow cytometry before and 6, 12, 18, 24 and 30 h following HBO exposure. Time point 0 represents pre-exposure. *P<0.05 compared with the pre-exposure level.

of HSP32, 60 70 and 90 in lymphocytes increased 2.4-, 1.4-, 1.9- and 1.2-fold, respectively (Fig. 2). The inducible HSP32 and 70 increased most significantly, with the greatest expression appearing at 18 h following HBO exposure; 18 h was therefore used as the interval from HBO exposure to simulated diving with the aim of measuring any beneficial effects of HBO pretreatment.

The results of our experiments showed that HBO pretreatment had effects (P<0.05) on bubble load, skin lesions and inflammatory and endothelial markers (see below), while the stage had no effect (P>0.05). Detailed statistical results are shown in Tables 1 and 2.

Table 1. Results of ANOVA with two-stage cross-over design data

Parameters	Sources of variation	F	P
Skin lesion area rate	Corrected model	19.903	0.000
	Subject	19.521	0.000
	Stage	0.131	0.726
	Pretreatment	43.115	0.000
Latency to stage III lesion	Corrected model	55.818	0.000
	Subject	60.667	0.000
	Stage	0.400	0.545
	Pretreatment	67.600	0.000
IL-8	Corrected model	17.601	0.000
	Subject	17.852	0.000
	Stage	0.619	0.454
	Pretreatment	32.325	0.000
MPC-1	Corrected model	7.185	0.005
	Subject	4.182	0.028
	Stage	0.602	0.460
	Pretreatment	40.789	0.000
MDA	Corrected model	23.081	0.000
	Subject	18.341	0.000
	Stage	3.827	0.086
	Pretreatment	85.002	0.000
ET-1	Corrected model	7.014	0.005
	Subject	3.806	0.037
	Stage	0.747	0.412
	Pretreatment	42.150	0.000
VCAM-1	Corrected model	5.472	0.012
	Subject	3.561	0.044
	Stage	0.003	0.960
	Pretreatment	28.146	0.001

IL-8, interleukine-8; MPC-1, monocyte chemoattractant protein-1; MDA, methane dicarboxylic aldehyde; ET-1, endothelin-1; VCAM-1, vascular cell adhesion molecule-1.

Table 2. Results of paired t-test and two independent-samples t-tests

Parameter	Pretreatments compared	P	Groups compared	P
Skin lesion area rate	Group 1	0.014	Air pretreatment	0.547
	Group 2	0.005	HBO pretreatment	0.588
	Combined	0.000	Combined	0.920
Latency to stage III lesion	Group 1	0.002	Air pretreatment	0.626
	Group 2	0.009	HBO pretreatment	0.784
	Combined	0.000	Combined	0.594
IL-8	Group 1	0.015	Air pretreatment	0.282
	Group 2	0.016	HBO pretreatment	0.103
	Combined	0.000	Combined	0.066
MPC-1	Group 1	0.015	Air pretreatment	0.966
	Group 2	0.008	HBO pretreatment	0.396
	Combined	0.000	Combined	0.686
MDA	Group 1	0.001	Air pretreatment	0.529
	Group 2	0.003	HBO pretreatment	0.900
	Combined	0.000	Combined	0.711
ET-1	Group 1	0.032	Air pretreatment	0.562
	Group 2	0.002	HBO pretreatment	0.975
	Combined	0.000	Combined	0.713
VCAM-1	Group 1	0.029	Air pretreatment	0.660
	Group 2	0.012	HBO pretreatment	0.550
	Combined	0.000	Combined	0.565

Bubble load following HBO pretreatment

Bubbles could be clearly observed in the right ventricular outflow tract and pulmonary artery in the ultrasound images. During the observation period, the number of bubbles was greatest at the first detection point, performed 30 min following decompression, and gradually decreased thereafter (Fig. 3A). HBO pretreatment reduced bubble load when compared with air pretreatment (P=0.000); the pretreatment order had no effect on bubble load (P=0.616; Fig. 3B).

Skin lesions following HBO pretreatment

Skin lesions indicative of DCS occurred in all swine after either of the simulated air dives. HBO pretreatment increased the latency to stage III lesion and reduced lesion surface area when compared with air pretreatment in both groups and when combined (P<0.01 or P<0.05; Fig. 4).

Changes in inflammatory indicators following HBO pretreatment

IL-8, MCP-1 and MDA gradually increased after the simulated dive and peaked at 6, 12 and 12 h, respectively (Fig. 5, left). The rate of change was compared between the two dives, the different pretreatments and the two groups at their peak time point. HBO pretreatment significantly reduced the increase in inflammatory indicators (P<0.05 or P<0.01).

Changes in endothelial markers following HBO pretreatment

The simulated dives induced a significant increase of serum ET-1 and VCAM-1, with the peak value appearing at 18 and 24 h, respectively. HBO pretreatment significantly reduced the levels of these markers at the respective peak time point (P<0.05 or P<0.01; Fig. 6).

DISCUSSION

Divers are at risk of DCS, a distinctive disorder caused by bubble generation following a rapid and extensive reduction of ambient pressure (Vann et al., 2011). The symptoms vary from mild

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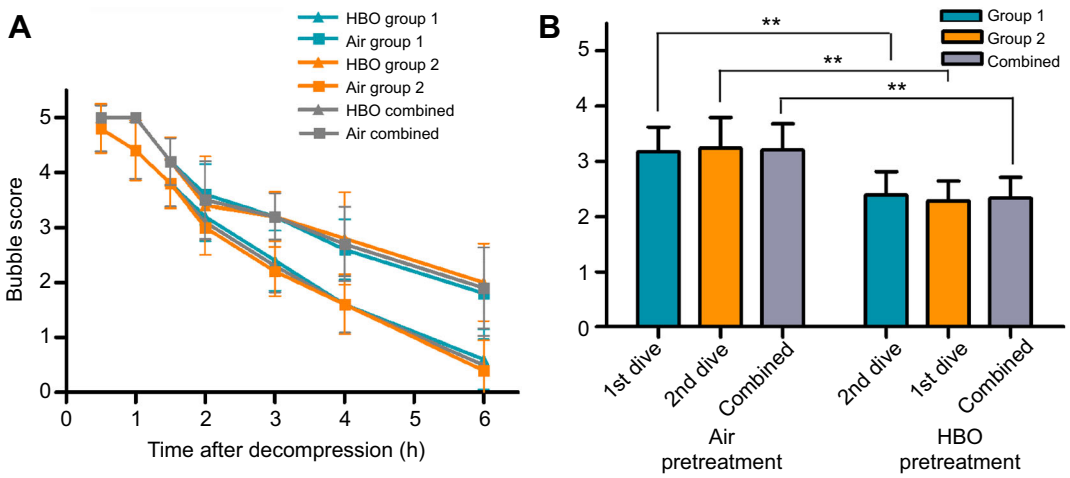

Fig. 3. Bubble load in swine after simulated diving following HBO pretreatment. Swine were equally divided into two groups (n=5 per group) and were subjected to two simulated dives following pretreatment with either air or HBO before each dive (see Fig. 1). Bubbles in heart chambers were detected by ultrasound at specific time points after decompression, and were scored by the Eftedal–Brubakk grading scale. (A) Bubble score plotted against time after decompression. (B) Bubble score according to pretreatment.

arthralgia and skin lesions to severe cardio-pulmonary or neurological function damage, and even sudden death (Vann et al., 2011). Invisible injuries, such as inflammatory responses and blood vessel endothelial injuries, also play a role in the progression of DCS (Brubakk and Møllerløkken, 2009; Levett and Millar, 2008; Vann et al., 2011). Immediate recompression at the nearest facility is the optimal treatment, which requires a well-equipped chamber, but this is absent in many cases. Reduction of the risk of DCS is vital.

Three distinct mechanisms have been proposed for the prophylactic action of HBO on DCS. The traditionally hypothesized mechanism is ‘denitrogenation’, which occurs immediately before exposure to a lower atmospheric pressure such as an EVA (Lambertsen, 1988). The ‘denucleation’ mechanism was proposed to explain the effects of HBO administered any time from several minutes to hours prior to a dive indicating a time interval is necessary between pretreatment and the subsequent hyperbaric exposure (Arieli et al., 2009). The third mechanism, in which HBO is pre-breathed more than 10–20 h prior to diving, seems to involve inducible proteins (Butler et al., 2006; Fan et al., 2010; Huang et al., 2014, 2016; Ni et al., 2013).

The present study was performed to test the third mechanism in a swine DCS model. In order to find the optimal time interval between HBO pre-exposure and DCS modeling, HSP expression following HBO exposure was studied first. Lymphocytes are an ideal blood cell type for flow-cytometric determination of induced HSPs (Cui et al., 2015), and the results showed that expression of all four determined HSPs increased after HBO exposure. Among these, HSP32 and 70 increased most significantly, with the greatest expression appearing at 18 h following HBO exposure, which is similar to results observed in rats in our previous study (Ni et al., 2013). Thus, 18 h was chosen as the interval for this experiment.

In order to minimize the number of animals used and to increase the efficiency of the study, a self-controlled experimental design

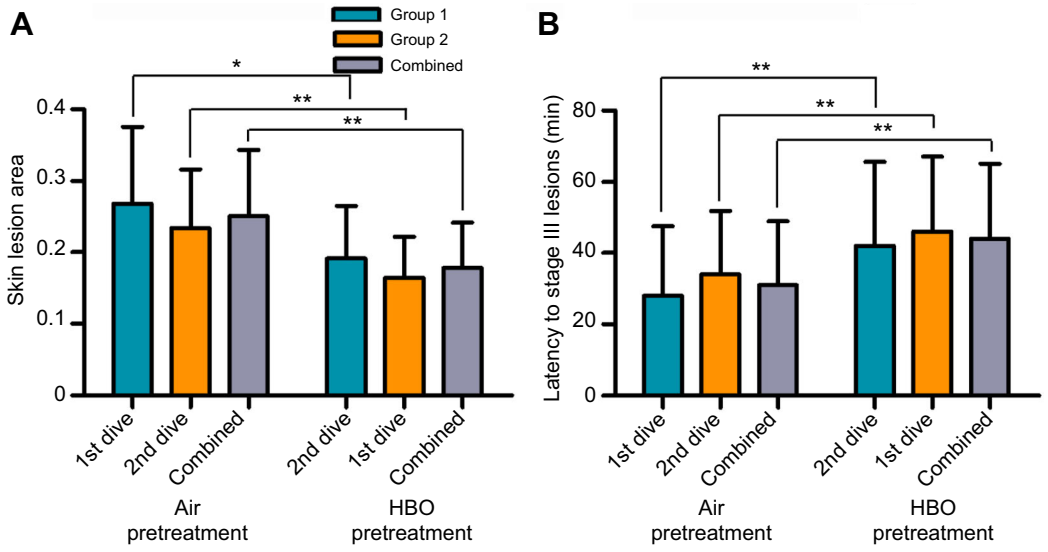

Fig. 4. Skin decompression sickness (DCS) lesions in swine after simulated diving following HBO pretreatment. Skin lesions were observed in both groups of swine (n=5 each) after simulated air dives following air or HBO pretreatment (see Fig. 1). The skin lesion area as a proportion of body surface area (A) and latency to stage III lesion (B) were compared between the two dives, the different treatments and the two groups. *P<0.05, **P<0.01.

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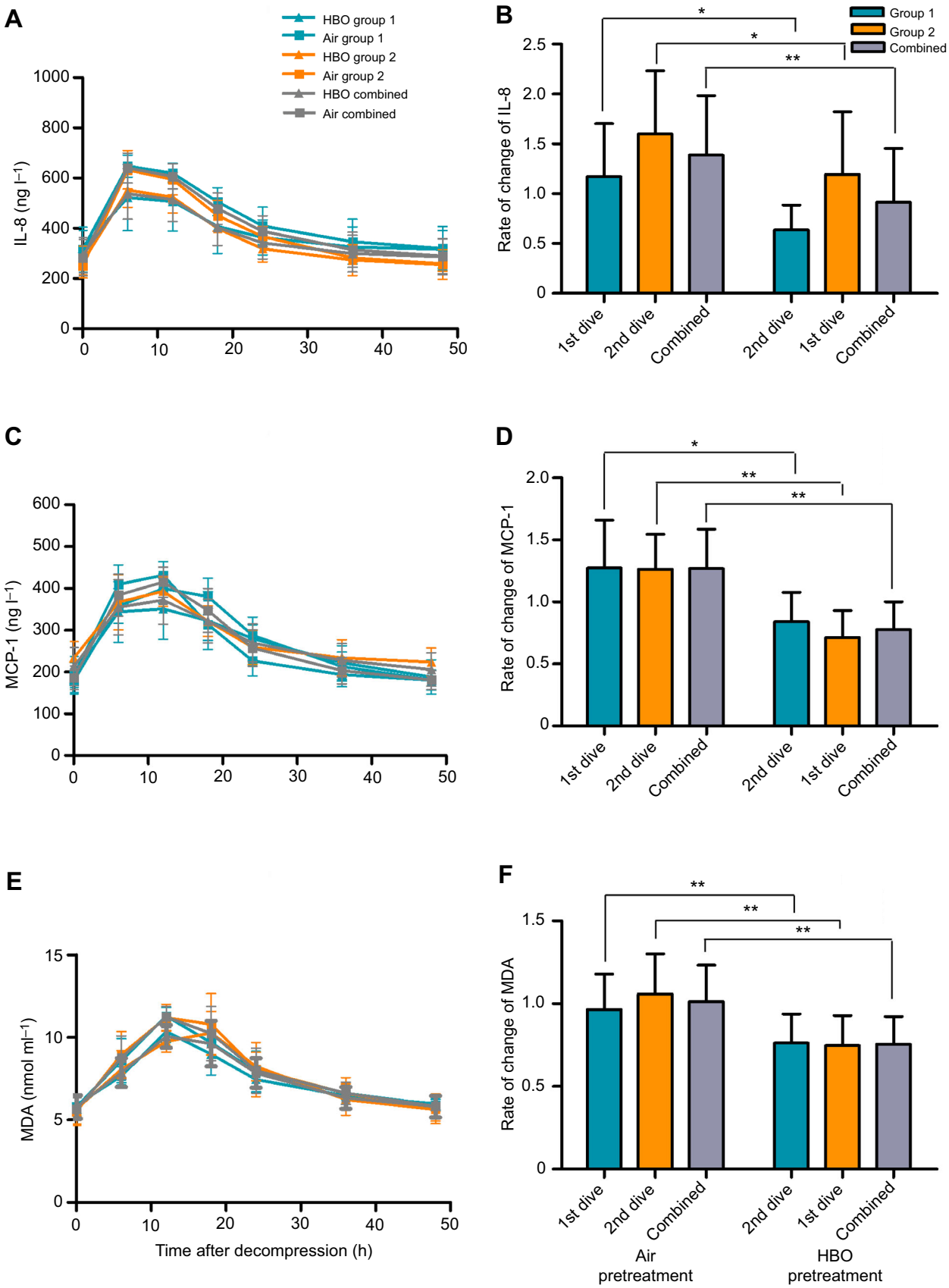

Fig. 5. Changes of inflammatory markers after simulated diving following HBO pretreatment. Blood was sampled from a venous catheter in swine pretreated with air or HBO before a simulated air dive and 6, 12, 18, 24, 36 and 48 h following decompression. (A,C,E) Interleukine-8 (IL-8; A), monocyte chemoattractant protein-1 (MCP-1; C) and methane dicarboxylic aldehyde (MDA; E) in serum were tested by ELISA. (B,D,F) The rate of change in IL-8, MCP-1 and MDA, respectively, was compared at the peak time point between the two dives, the different treatments and the two groups. *P<0.05, **P<0.01.

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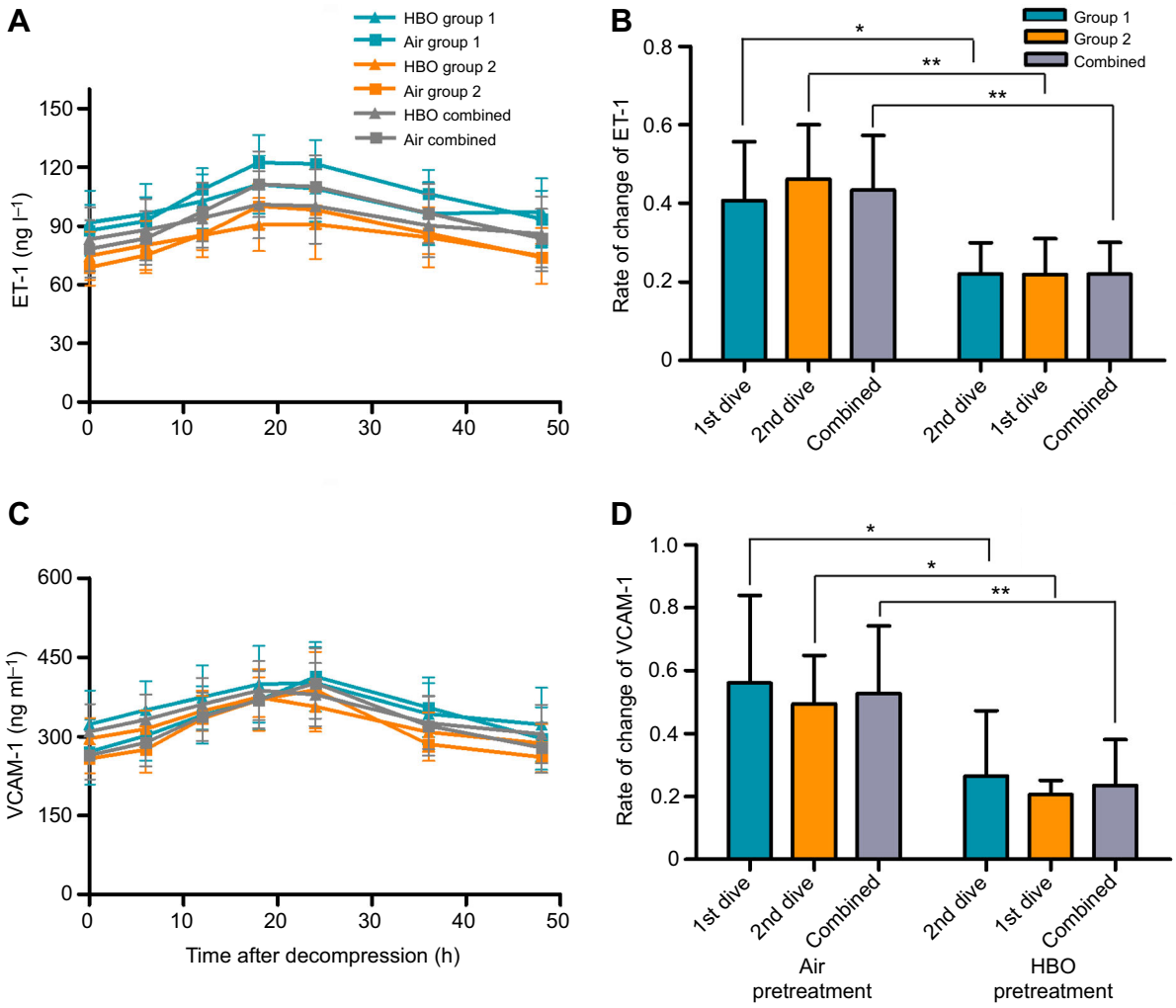

Fig. 6. Changes of endothelial markers following HBO pretreatment. Blood samples were obtained from a venous catheter in swine pretreated with air or HBO before a simulated air dive and at 6, 12, 18, 24, 36 and 48 h following decompression. (A,C) Serum endothelin-1 (ET-1; A) and vascular cell adhesion molecule-1 (VCAM-1; C) levels were tested by ELISA. (B,D) The rate of change in ET-1 and VCAM-1, respectively, was compared at the peak time points between the two dives, the different treatments and the two groups. *P<0.05, **P<0.01.

was adopted. Each animal underwent the compression/decompression protocol twice, following pretreatment with HBO or normobaric air. With regard to the potential impact of repetitive diving on DCS, with the resulting possible increase or decrease in DCS risk (Rhind et al., 2007; Vann, 1989), a prolonged 7 day interval was adopted and the swine were divided into two groups with different pretreatment sequences of HBO or air to investigate the possible influence of the first dive on the second. From the results, no significant differences were found between the outcomes of the first and second dive in the swine, whether pretreated with air or with HBO, or when combining the two groups as a whole (P>0.05, data not shown). All results in this study suggest that the differences come from the different pretreatments.

The current experimental results clearly demonstrate that HBO pretreatment ameliorates the symptoms of DCS. Neurological change was not found in our previous experiment using the same animal model (Qing et al., 2017), so it was not determined in this study, and therefore the potential benefits of HBO pretreatment on neurological DCS remain to be investigated.

The beneficial effects of HBO pretreatment on DCS were postulated to result from the induction of HSPs. HBO exposure is considered to be a moderate oxidative stress, which could increase the production of reactive oxygen species in animals including swine. Reactive oxygen species serve as signaling molecules in inducing the expression of protective proteins including HSPs, which play a leading role in HBO preconditioning in various disorders (Thom, 2011). Among the HSP family, HSP27, HSP32, HSP60, HSP70 are the main inducible members, and contribute substantially to maintaining homeostasis during disease or injuries (Latchman, 2001; Maines, 1997; Redaelli et al., 2001; Wiechmann et al., 2017). Oxidative stress and ischemia–reperfusion are crucial in the etiology of DCS (Vann et al., 2011). HSPs directly interfere with oxidative injury and ischemia-like insults by reducing alterations in cellular redox status in endothelial cell survival/death pathways (Djurhuus et al., 2010; Yenari et al., 2005). In our previous studies *in vivo* and *in vitro*, HSP32 and HSP70 increased significantly following HBO exposure and were preventive for DCS and oxidative/oxygen–glucose deprivation insults, respectively (Huang et al., 2014, 2016; Ni et al., 2013). HSP32, also known as heme oxygenase-1, is one of the rate-limiting enzymes in heme catabolism, which leads to the generation of ferrous iron, biliverdin and CO, with anti-oxidative, anti-inflammatory and anti-apoptotic consequences (Maines, 1997). HSP70 is linked to less apoptotic cell death, which is associated with endothelial protection during DCS

(Djurhuus et al., 2010). Furthermore, HSP70 could suppress monocyte activation to impair proinflammatory cytokine production (Yenari et al., 2005). As the key mitochondrial molecular chaperone, HSP60 is especially required under cellular stress conditions, and its expression is increased in response to stress stimuli (Kim et al., 2017). For HSP90, although it is a constitutive protein, in many circumstances it is also inducible and regulates numerous client proteins to counteract various injuries (Latchman, 2001; Redaelli et al., 2001). HSPs were considered to play a role in reducing neurological DCS in rabbits that received heat exposure prior to dives (Su et al., 2004). However, HSP27 did not change significantly after HBO treatment in rats and in neurons *in vitro* (Huang et al., 2014; Ni et al., 2013). To produce mild oxidative stress that was nonetheless sufficient to induce HSP expression, HBO higher than a certain pressure (>2 atmospheres absolute) may be warranted.

The induction of HSPs may also be the cause of decreased bubble formation as a result of the beneficial effects of HSPs on endothelial cells, which may be the generating sites of microbubbles (Butler et al., 2006). However, the 'denucleation' etiology of HBO pretreatment might also be involved in the reduction of bubbles, as the generation of gas nuclei in the body takes 10–100 h (Yount, 1982). The generation of gas nuclei may be related to the heart and respiratory rates, which are several times higher in rats than in swine. This might be a possible explanation for the results of our previous study, in which no change in bubble formation was detected following HBO pretreatment in a rat DCS model (Fan et al., 2010). The exact causes of reduced bubble formation deserve further study.

In our previous rat and *ex vivo* neuron experiments, a control group using HSP inhibitors was adopted to verify the involvement of HSPs. Because of limits to drug administration to swine, the effects of HSP inhibition were not observed. Therefore, the precise role of HSPs in the protective effect of HBO pretreatment is mostly speculative in this study. Other inducible protective proteins such as hypoxia-inducible factor and vascular endothelial growth factor may also be involved (Thom, 2011).

Studying the time course of injuries will help to better elucidate the pathophysiological process of decompression stress and this is of great significance in clinical diagnosis, as divers suffering from DCS are frequently delay-treated (Vann et al., 2011). From the curves of combined air pretreatment presented in Figs 5 and 6, it can be seen that the duration of half-maximal elevation levels for IL-8, MCP-1, MDA, ET-1 and VCAM-1 occurred around 3–18 h, 3–20 h, 6–22 h, 12–36 h and 12–33 h, respectively. These changes were similar to those found in our previous rat DCS study (Zhang et al., 2017). From the current results and those we have acquired in rats, it can be speculated that the endothelial indices are ideal biomarkers in diagnosing decompression stress.

Taken together, this is the first study to reveal that HBO pretreatment more than 10 h prior to diving significantly reduces the risk of DCS in a swine model. HSP induction might be the underlying mechanism. Discerning the time course of pathophysiological biomarkers would help in the clinical diagnosis of DCS. As a routine operation in diving and hyperbaric activities, HBO breathing independently or integrated into hyperbaric exercise before diving is convenient and practicable, and will be a valuable approach in decreasing DCS risk. Further research in divers is warranted.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: W.X., L.Q.; Methodology: W.X., L.Q., H.Y., Y.W., Q.Z., D.K.A.; Formal analysis: L.Q., H.Y., D.K.A.; Investigation: L.Q., Y.W., Q.Z., D.K.A.; Resources: W.X.; Writing - original draft: W.X., L.Q., H.Y., Y.W., D.K.A.; Writing - review & editing: W.X., L.Q., H.Y.; Funding acquisition: W.X.

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References
Arieli, R., Boaron, E. and Abramovich, A. (2009). Combined effect of denucleation and denitrogenation on the risk of decompression sickness in rats. *J. Appl. Physiol.* **106**, 1453-1458.

Brubakk, A. O. and Møllerløkken, A. (2009). The role of intra-vascular bubbles and the vascular endothelium in decompression sickness. *Diving Hyperb Med.* **39**, 162-169.

Butler, B. D., Little, T., Cogan, V. and Powell, M. (2006). Hyperbaric oxygen pre-breathe modifies the outcome of decompression sickness. *Undersea Hyperb. Med.* **33**, 407-417.

Cui, X., Xing, J., Liu, Y., Zhou, Y., Luo, X., Zhang, Z., Han, W., Wu, T. and Chen, W. (2015). COPD and levels of Hsp70 (HSPA1A) and Hsp27 (HSPB1) in plasma and lymphocytes among coal workers: a case-control study. *Cell Stress Chaperones* **20**, 473-481.

Djurhuus, R., Nossum, V., Lundsett, N., Hovin, W., Svardal, A. M., Havnes, M. B., Fismen, L., Hjelde, A. and Brubakk, A. O. (2010). Simulated diving after heat stress potentiates the induction of heat shock protein 70 and elevates glutathione in human endothelial cells. *Cell Stress Chaperones* **15**, 405-414.

Eftedal, O. and Brubakk, A. O. (1997). Agreement between trained and untrained observers in grading intravascular bubble signals in ultrasonic images. *Undersea Hyperb. Med.* **24**, 293-299.

Fan, D. F., Liu, K., Xu, W. G., Zhang, R. J., Liu, Y., Kang, Z. M., Sun, X. J., Li, R. P., Tao, H. Y. and Zhang, J. L. (2010). Hyperbaric oxygen preconditioning reduces the incidence of decompression sickness in rats via nitric oxide. *Undersea Hyperb. Med.* **37**, 173-180.

Huang, G., Xu, J., Xu, L., Wang, S., Li, R., Liu, K., Zheng, J., Cai, Z., Zhang, K., Luo, Y. et al. (2014). Hyperbaric oxygen preconditioning induces tolerance against oxidative injury and oxygen-glucose deprivation by up-regulating heat shock protein 32 in rat spinal neurons. *PLoS ONE* **9**, e85967.

Huang, G., Diao, J., Yi, H., Xu, L., Xu, J. and Xu, W. (2016). Signaling pathways involved in HSP32 induction by hyperbaric oxygen in rat spinal neurons. *Redox Biol.* **10**, 108-118.

Kim, B. Y., Son, Y., Choi, J., Eo, S. K., Park, Y. C. and Kim, K. (2017). 27-Hydroxycholesterol upregulates the production of heat shock protein 60 of monocytic cells. *J. Steroid Biochem. Mol. Biol.* **172**, 29-35.

Lambertsen, C. J. (1988). Extension of oxygen tolerance in man: philosophy and significance. *Exp. Lung Res.* **14**, 1035-1058.

Latchman, D. S. (2001). Heat shock proteins and cardiac protection. *Cardiovasc. Res.* **51**, 637-646.

Levett, D. Z. and Millar, I. L. (2008). Bubble trouble: a review of diving physiology and disease. *Postgrad. Med. J.* **84**, 571-578.

Maines, M. D. (1997). The heme oxygenase system a regulator of second messenger gases. *Annu. Rev. Pharmacol. Toxicol.* **37**, 517-554.

Ni, X.-X., Ni, M., Fan, D.-F., Sun, Q., Kang, Z.-M., Cai, Z.-Y., Liu, Y., Liu, K., Li, R.-P. and Xu, W.-G. (2013). Heat-shock protein 70 is involved in hyperbaric oxygen preconditioning on decompression sickness in rats. *Exp. Biol. Med.* **238**, 12-22.

Qing, L., Dinesh, K. A., Yi, H., Wang, Y., Zhou, Q. and Xu, W. (2017). Skin lesions in swine with decompression sickness: clinical appearance and pathogenesis. *Front. Physiol.* **8**, 540.

Quiring, D. P. (1955). Surface area determination. In *Medical Physics*, **Vol. 1** (ed. O. Glasser), pp. 1490-1494. Chicago: Yearbook.

Redaelli, C. A., Wagner, M., Kulli, C., Tian, Y.-H., Schilling, M. K., Wagner, A. C. C., Mazzucchelli, L. and Kubulus, D. (2001). Hyperthermia-induced HSP expression correlates with improved rat renal isograft viability and survival in kidneys harvested from non-heart-beating donors. *Transpl. Int.* **14**, 351-360.

Rhind, S. G., Cameron, B. A. and Eaton, D. J. (2007). Heat shock protein 70 is upregulated in leukocytes from experienced divers in response to repetitive hyperbaric stress. Abstract of the Undersea and Hyperbaric Medical Society, Inc. Annual Scientific Meeting, Ritz-Carlton Kapalua Maui, Hawaii, 14-16.

Russell, W. B. R., Burch, R. L. and Hume, C. W. (1959). *The Principles of Humane Experimental Technique*. London: Methuen.
Su, C.-L., Wu, C. P., Chen, S. Y., Kang, B. H., Huang, K. L. and Lin, Y. C. (2004). Acclimatization to neurological decompression sickness in rabbits. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **287**, R1214-R1218.
Thom, S. R. (2011). Hyperbaric oxygen: its mechanisms and efficacy. *Plast. Reconstr. Surg.* **127**, 131S-141S.
Vann, R. D.(ed.) (1989). The effect of pressure profile on bubble formation. In *The Physiological Basis of Decompression*. 38th Undersea and Hyperbaric Medical Society Workshop. UHMS Publication Number 75(Phys)6-1-89, 437pp. Bethesda: Undersea and Hyperbaric Medical Society.
Vann, R. D., Butler, F. K., Mitchell, S. J. and Moon, R. E. (2011). Decompression illness. *Lancet* **377**, 153-164.
Weaver, L. K. (2014). *Undersea and Hyperbaric Medical Society Hyperbaric Oxygen Therapy Indications*, 13th edn. North Palm Beach, FL: Best Publishing.
Wiechmann, K., Müller, H., König, S., Wielsch, N., Svatoš, A., Jauch, J. and Werz, O. (2017). Mitochondrial chaperonin HSP60 is the apoptosis-related target for myrtucommulone. *Cell Chem. Biol.* **24**, 614-623.e6.
Yenari, M. A., Liu, J., Zheng, Z., Vexler, Z. S., Lee, J. E. and Giffard, R. G. (2005). Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. *Ann. N. Y. Acad. Sci.* **1053**, 74-83.
Yount, D. (1982). On the evolution, generation and regeneration of gas cavitation nuclei. *J. Acoust. Soc. Am.* **71**, 1473-1481.
Zhang, K., Wang, M., Wang, H., Liu, Y., Buzzacott, P. and Xu, W. (2017). Time course of endothelial dysfunction induced by decompression bubbles in rats. *Front. Physiol.* **8**, 181.